Original Article

Efficacy of Hyaluronic acid against Cyclophosphamide Toxic Effects on the Urinary Bladder of Adult Male Albino Rat (Histological and Immunohistochemical Study)

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ABSTRACT

Introduction: Cyclophosphamide (CP) is a widely used potent antineoplastic drug. Cyclophosphamide has many genitourinary side effects including cystitis. Hyaluronic acid (HA) is used in many types of cystitis as ketamine induced cystitis and interstitial cystitis as it is known to be well-tolerated and clinically safe.

Aim of the Work: This work aimed to evaluate the role of intravesical injection of hyaluronic acid in ameliorating cyclophosphamide toxic effects on the urinary bladder of adult male albino rats.

Material and Methods: Forty adults males albino rats were used, ages from 6-8 month, weighing from 200 -250 gm. Rats were randomly divided into equal four groups:

Group I (Control group): Rats of this group were further subdivided equally into:

- Ia: rats received food and water only for 14 days.

- Ib: each rat was intraperitoneally injected with 0.5 ml saline daily for 14 days. Group II (HA group): each rat was intravesically injected with 0.5 mL hyaluronic acid daily for 14 days. Group III (CP group): each rat was intraperitoneally injected with 30 mg/kg/day of cyclophosphamide for 14 days. Group IV (HA+ CP group): each rat was intraperitoneally injected with cyclophosphamide as in group III and intravesically injected with hyaluronic acid as in group II.

Results: Cyclophosphamide administration induced marked histological changes of rats' urinary bladders in the form of lost mucosal folds, focal ulcerations, inflammatory reaction in the lamina propria and decreased collagen content while, administration of cyclophosphamide concomitant with intravesical injection of hyaluronic acid showed regular histological structure of rats' urinary bladders, intact mucosa, slight inflammation of lamina propria and proper amount of collagen fibers.

Conclusion: Intravesical injection of hyaluronic ameliorates cyclophosphamide toxic effects on the urinary bladder of adult male albino rats.

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Key Words: Cyclophosphamide, cystitis, hyaluronic acid.

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INTRODUCTION

The urinary bladder is a hollow distensible muscular organ, its function involves urine storage and periodic urine evacuation. The bladder epithelium has slow turnover (3-6 months) in comparison to epidermis and gut epithelium (1.5-30 days)[1,2]. Cystitis means urinary bladder inflammation which occurs by several factors, such as infection, drugs and radiation[3].

Cyclophosphamide (CP) is an alkylating agent of oxazaphosphorine class. It is a widely used potent antineoplastic drug in common malignancies such as breast cancer, lymphoma and leukemia. It is also an efficient immunosuppressive drug in autoimmune diseases. Its toxic effects on the cells are achieved through alkylation of guanine groups of DNAs which prevents the double strands from uncoiling and separation with subsequent prevention of cellular multiplication followed by cellular apoptosis. This type of drugs affects both cycling and resting cells with higher toxicity for the actively dividing cells[4,5].

Cyclophosphamide has many genitourinary side effects including disturbance in gonadotropin secretion, testicular damage and cystitis. It increases the production of free radicals leading to DNA damage and decreases glutathione level. Therefore, adding cyclophosphamide to a powerful antioxidant is thought to be an appropriate method to decrease its side effects[6-10].

Hyaluronic acid (HA) consists of repetitive disaccharides of glucuronic acid and N-acetylglucosamine. It is a natural polysaccharide which forms crucial constituents of extracellular matrix, approximately in all living tissues. In addition to its well-known lubricating and healing properties, it has anti-inflammatory effect and

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antioxidant effect against cellular oxidative damage. Hence, it is used in many types of cystitis as ketamine induced cystitis, radiation induced cystitis, interstitial cystitis and recurrent bacterial cystitis. Exogenous hyaluronic acid administration is known to be well-tolerated and clinically safe[11-13].

Therefore, this study was conducted to evaluate the role of intravesical injection of hyaluronic acid in ameliorating cyclophosphamide toxic effects on the urinary bladder of adult male rats.

MATERIALS AND METHODS

Chemicals

- Cyclophosphamide (Endoxan) was obtained in the form of dry powder (200 mg) in a vial (Baxter Company, Deerfield, IL, USA). Each vial was dissolved in 10 ml saline.
- Hyaluronic acid (Cystistat) was obtained in the form of sterile solution 50ml (Mylan, Canonsburg, PA).

The procedure of intravesical injection

A sterile polyethylene tube (PE-50; Clay-Adams, Parsippany, NJ) has been inserted into the bladder through the urethra, after anesthesia by ether inhalation. The urine was aspirated then hyaluronic acid was injected via 1 mL syringe along the tube which kept in the bladder for 30 mins following injection[14].

Animals

Forty albino rats (adults-males) were used, ages from 6-8 month, weighing from 200 -250 gm. Rats were obtained and kept at Medical Research Center, Faculty of Medicine, Ain-Shams University. They were retained in metal cages having good ventilation and subjected to regular dark-light cycles at room temperature. food and water were freely allowed.

Rats were randomly divided into four equal groups (10 rats in each group):

Group I (Control group): Rats of this group were subdivided into two equal subgroups:

- Ia: rats received food and water only for 14 days.
- Ib: each rat was intraperitoneally injected with 0.5 ml saline daily for 14 days.

Group II (HA group): each rat was intravesically injected with 0.5 mL hyaluronic acid daily for 14 days[14].

Group III (CP group): each rat was intraperitoneally injected with 30 mg/kg/day of cyclophosphamide for 14 days[15].

Group IV (HA+ CP group): each rat was intraperitoneally injected with cyclophosphamide as in group III and intravesically injected with hyaluronic acid as in group II.

The Ethical committee approval No. for this study is FMASU R45/2023, Faculty of Medicine, Ain Shams University.

Processing of samples

Rats were sacrificed under anesthesia (inhalation of diethyl ether). The anterior abdominal wall was dissected, and the urinary bladder of each rat was obtained then washed with saline. Each urinary bladder has been longitudinally split into two parts; the first part was fixed in neutral-buffered formalin (10%). The second part was cut into small pieces then fixed in glutaraldehyde (2.5%). Then paraffin and epon blocks were formed respectively. Paraffin sections (5μm thickness) were stained with Hematoxylin and eosin (H. & E.) for the standard histological examination[16] other sections were stained with Masson's trichrome stain to clarify the collagen fibers[17]. For immunohistochemical staining, deparaffinized sections were cut and mounted on positively charged slides for:

1. Nuclear factor kappa B (NF-κB) "indicator of inflammation" (Polyclonal rabbit antibody (1:200), Labvision, Fremont, California, USA). Positive immune reaction appeared brown cytoplasmic staining.
2. Proliferating cell nuclear antigen (PCNA) "indicator of cellular proliferation" (Monoclonal anti-PCNA IgG (1:200), Sigma-Aldrich Inc., U.K.). Positive immune reaction appeared brown nuclear staining.

Sections were counterstained with hematoxylin. In negative controls, the primary antibody was replaced by phosphate-buffered saline. Positive controls for NF-κB were sections from prostatic carcinoma and for PCNA were sections from skin[18].

For semithin sections, 1μm sections from epon blocks were cut by LKB. Ultra-Microtome and stained with Toluidine blue[19].

Sections were examined and photographed by Olympus light microscope with an Automatic Photomicrographic Camera (BX3M series, Olympus, Tokyo, Japan) at Anatomy Department, Faculty of Medicine, Ain Shams University.

Morphometric analysis

Morphometric analysis was achieved using Image-J software (version 1.48v National Institute of Health, Bethesda, Maryland, USA). Ten non-overlapping fields in ten different sections from the ten rats of the same group were used for measuring the mean epithelial thickness in μm, mean area % of collagen, mean area % of NF-κB and PCNA immunoreactivity. The magnification used for measuring collagen and NF-κB was x400, x100 for PCNA and x40 for the epithelial thickness. Pixels were scaled for actual measurements using a stage micrometer.

Statistical analysis

SPSS software (version 20, IBM Corp., Armonk, NY, USA), one-way ANOVA and Bonferroni Post Hoc test were use. Differences between rats' groups were compared. Data were presented as the mean value ± standard deviation. Differences were nonsignificant with the *P-value* > 0.05, highly significant with the *P-value* ≤ 0.001 and significant with the *P-value* ≤ 0.05.

RESULTS

H. & E. stain and semithin sections

Examination of group I (Ia and Ib) and group II urinary bladders showed the same regular structure; highly folded mucosa with transitional epithelium, the epithelial cells were tightly packed (dome-shaped cells (superficial layer), pear-shaped cells (intermediate layer) then the basal cells). The cells were having rounded to oval vesicular nuclei and resting on a well identified intact corrugated basement membrane, followed by a vascular lamina propria. The smooth muscles layer underlying the lamina propria formed of muscle bundles running in various directions and separated by minimal connective tissue with their cells having oval nuclei (Figures 1-4).

Group III (CP group) showed areas of mucosal flattening, focal loss of epithelial continuity and decreased epithelial thickness. Most of the epithelial cells of all types showed small deeply stained irregular nuclei with underlying hardly identified basement membrane. The lamina propria appeared extensively infiltrated with multiple blood vessels and inflammatory cells. Cellular debris were noticed in the lumen. The smooth muscle bundles were widely separated with some cells having irregular nuclei. (Figures 5-8).

Group IV (CP+HA group) showed folded intact mucosa with tightly packed epithelial cells with vesicular nuclei and well identified intact corrugated underlying basement membrane. The lamina propria appeared slightly increased with few dilated blood vessels and few inflammatory cells. The smooth muscle bundles appeared separated by minimal connective tissue with their cells having oval nuclei (Figures 9-12).

Masson trichrome stained sections

Group I, group II and IV showed almost the same amount of collagen fibers in the lamina propria and between the muscle bundles. Group III showed apparent decrease of collagen fibers in the lamina propria and between the muscle bundles (Figures 13-15).

Nuclear factor kappa B immunohistochemically stained sections

Group I and group II showed negative cytoplasmic immune reaction of most epithelial cells. Group III showed strong cytoplasmic immune reaction of most epithelial cells and group IV showed negative cytoplasmic immune reaction of most epithelial cells. However, very few cells showed weak positive immune reaction (Figures 16-18).

Proliferating cell nuclear antigen (PCNA) immunohistochemically stained sections

Group I, group II and group IV showed positive immune reaction of sparse basal nuclei of epithelial cells. Group III showed positive immune reaction of multiple nuclei among the epithelium (Figures 19-21)

Morphometric results and statistical analysis

Morphometric measures for the mean epithelial thickness in µm, mean area % of collagen, NF-κB and PCNA immunoreactivity of the groups I, III and IV were displayed in Table I and Histogram I. The statistical difference was highly significant between group I and III and between group III and IV for all measurements. On other hand, the statistical difference was not significant between group I and IV for all measurements.

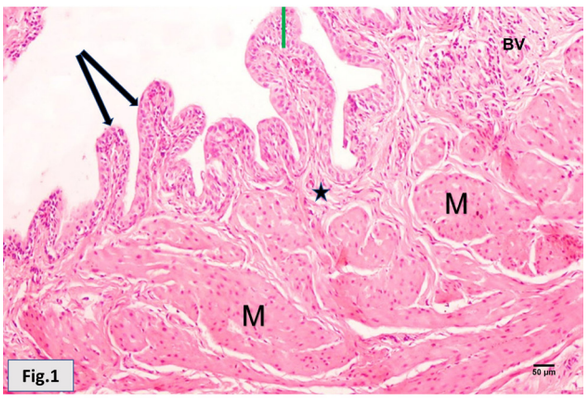


Fig. 1: A photomicrograph of group I rat's urinary bladder section showing highly folded mucosa (black arrows), transitional epithelium thickness (green line), the underlying lamina propria (*) with small blood vessels (BV) and the smooth muscle bundles running in various directions (M). (H. &E., x40- Scale bar,50µm)

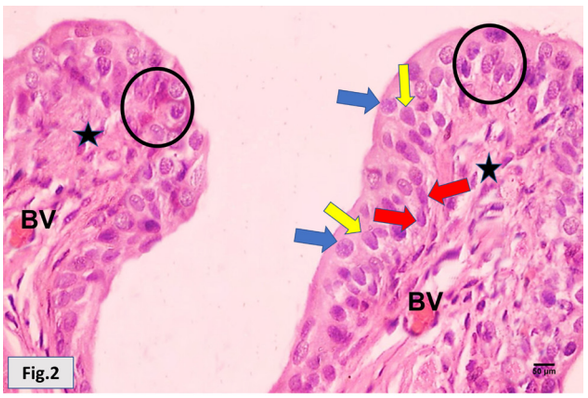


Fig. 2: A photomicrograph of group I rat's urinary bladder section showing tightly packed epithelial cells (circles); superficial dome-shaped cells (blue arrows), pear-shaped cells (yellow arrows) and basal cells (red arrows) and lamina propria (*) with small blood vessels (BV). (H. &E., x400-Scale bar,50µm)

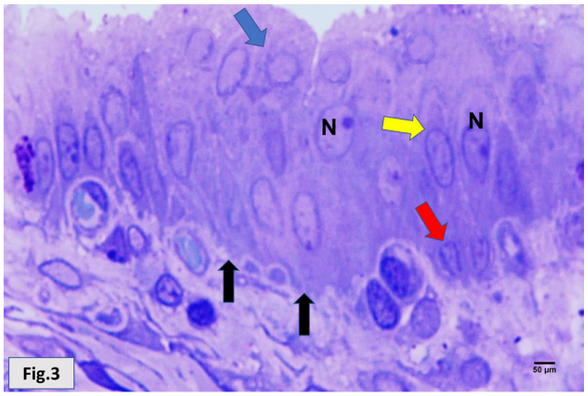


Fig. 3: A photomicrograph of group I rat's urinary bladder semithin section showing the epithelial cells; dome-shaped cells (blue arrow), pear-shaped cells (yellow arrow) and basal cells (red arrow). Most of the cells having rounded to oval vesicular nuclei (N) and resting on a well identified corrugated intact basement membrane (black arrows). (Toluidine blue, x1000, Scale bar,50µm)

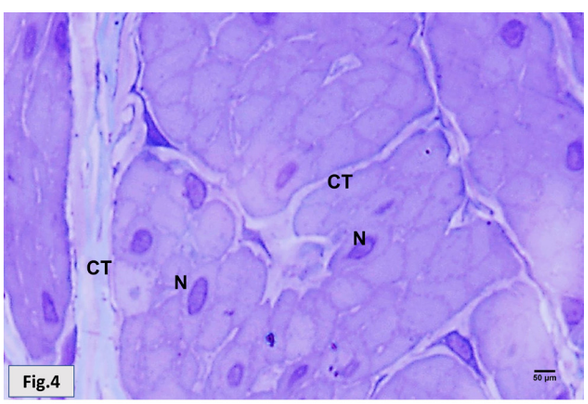


Fig. 4: A photomicrograph of group I rat's urinary bladder semithin section showing smooth muscle bundles separated by minimal connective tissue (CT) with their cells having oval nuclei (N). (Toluidine blue, x1000-Scale bar,50µm)

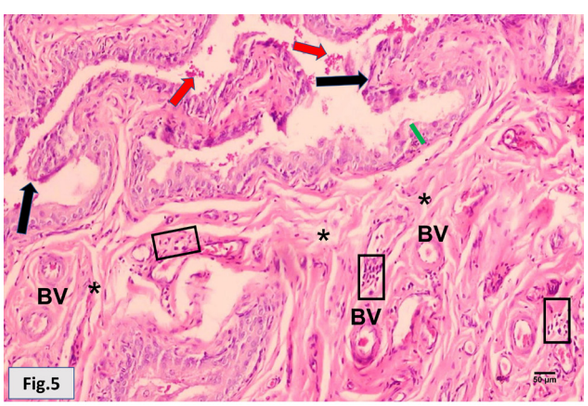


Fig. 5: A photomicrograph of group III rat's urinary bladder section showing focal loss of epithelial continuity (Black arrows), decreased epithelial thickness (green line). Wide lamina propria (*) with multiple blood vessels (BV) and inflammatory cells (rectangles). Notice, the cellular debris in the lumen (red arrow). (H. &E., x40-Scale bar,50µm)

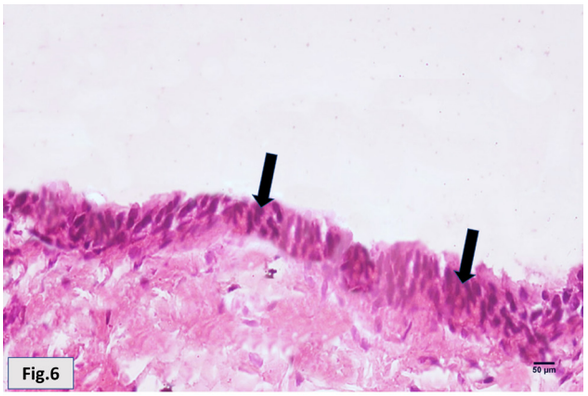


Fig. 6: A photomicrograph of group III rat's urinary bladder section showing an area with lost mucosal folds and the epithelial cells of all layers having deeply stained nuclei (Black arrows). (H. &E., x400-Scale bar,50µm)

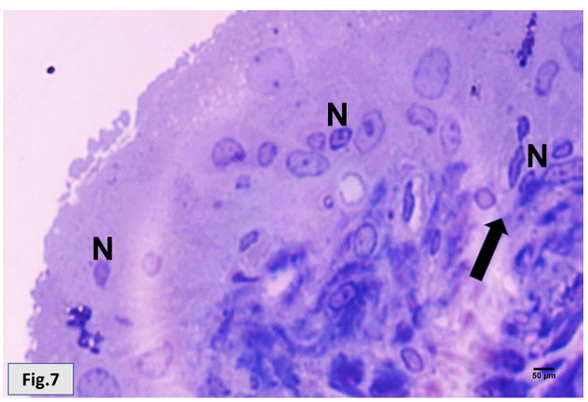


Fig. 7: A photomicrograph of group III rat's urinary bladder semithin section showing the epithelial cells having small dark irregular nuclei (N) with hardly identified underlying basement membrane (black arrow). (Toluidine blue, x1000-Scale bar,50µm)

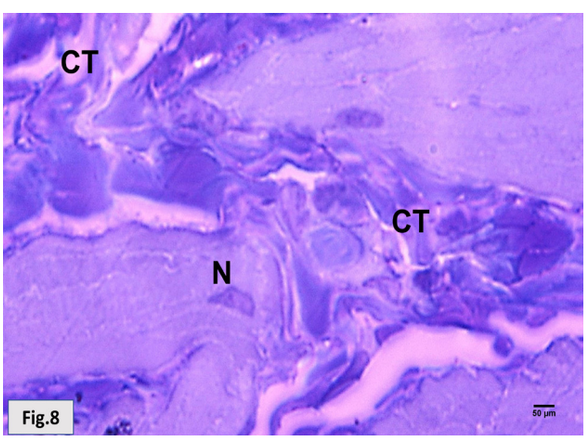


Fig. 8: A photomicrograph of group III rat's urinary bladder semithin section showing widely separated smooth muscle bundles with an irregular nucleus (N). (Toluidine blue, x1000-Scale bar,50µm)

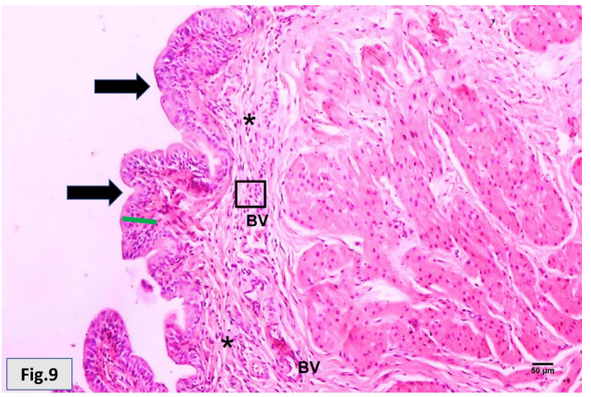


Fig. 9: A photomicrograph of group IV rat's urinary bladder section showing intact folded mucosa (black arrows) with transitional epithelium (green line). The lamina propria appeared slightly increased (*) with few dilated blood vessels (BV) and few inflammatory cells (rectangle) (H. &E., x40-Scale bar,50µm)

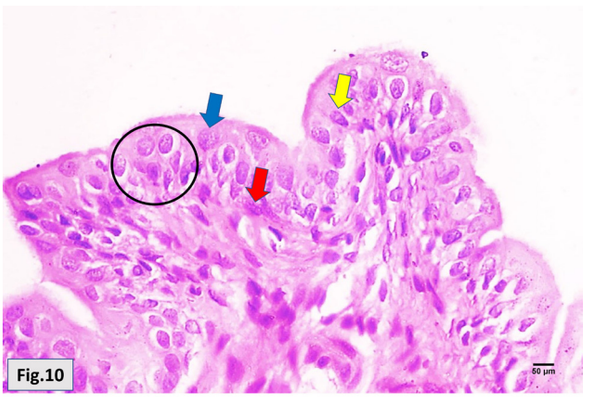


Fig. 10: A photomicrograph of group IV rat's urinary bladder section showing tightly packed epithelial cells (circle); dome-shaped cells (blue arrows), pear-shaped cells (yellow arrows) and basal cells (red arrows). (H. &E., x400-Scale bar,50µm)

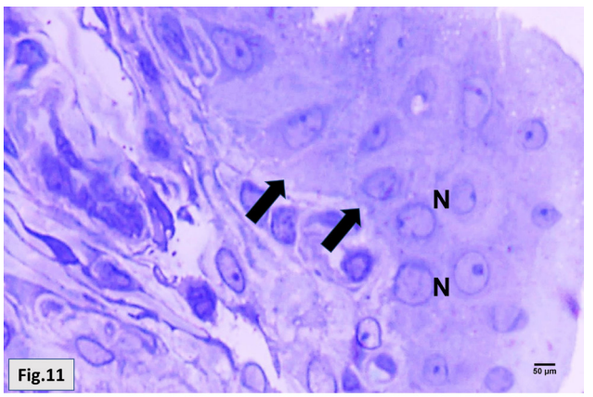


Fig. 11: A photomicrograph of group IV rat's urinary bladder semithin section showing the epithelial cells having mainly rounded to oval vesicular nuclei (N) resting on well identified intact corrugated basement membrane (black arrows). (Toluidine blue, x1000-Scale bar,50µm)

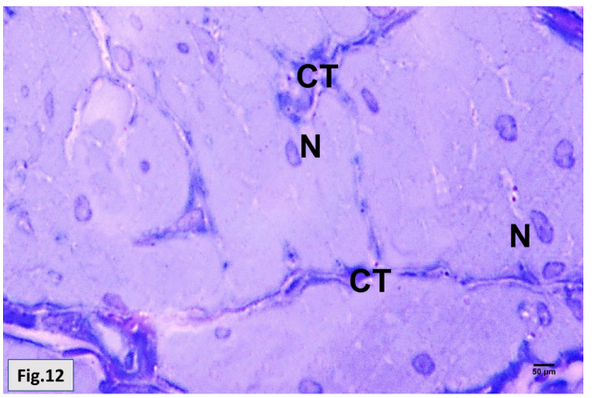


Fig. 12: A photomicrograph of group IV rat's urinary bladder semithin section showing smooth muscle bundles separated by minimal connective tissue (CT) with their cells having oval nuclei (N) (Toluidine blue, x1000-Scale bar,50µm)

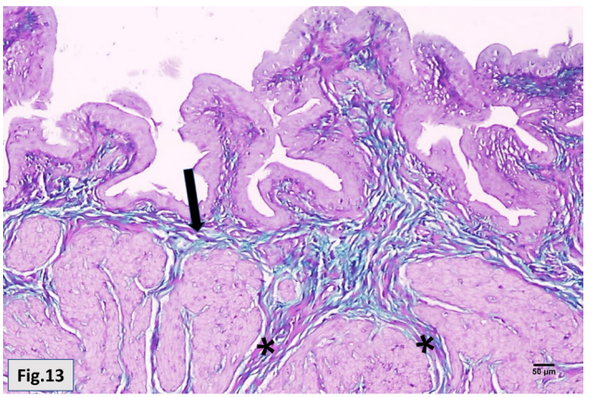


Fig. 13: A photomicrograph of group I rat's urinary bladder section showing the collagen fibers in the lamina propria (Black arrow) and in-between the muscle bundles (*). (Masson's trichrome ×40-Scale bar,50µm)

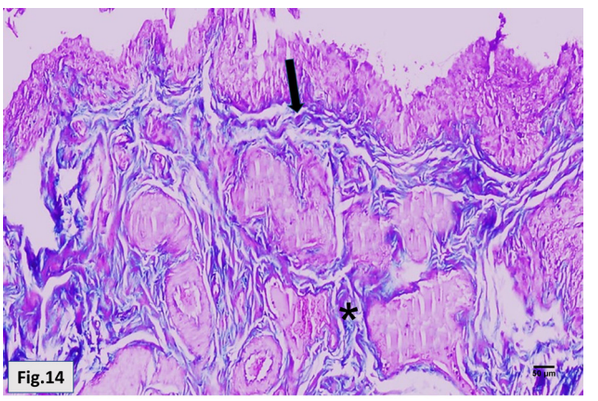


Fig. 14: A photomicrograph of group III rat's urinary bladder section showing apparent decrease in collagen fibers in the lamina propria (Black arrow) and in-between the muscle bundles (*). (Masson's trichrome ×40-Scale bar,50µm)

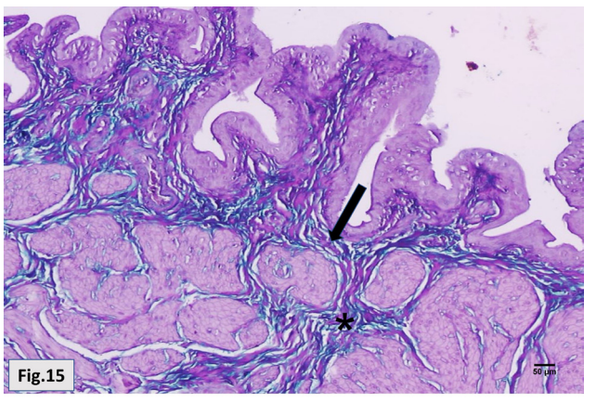


Fig. 15: A photomicrograph of group IV rat's urinary bladder section showing apparent average amount of collagen fibers in the lamina propria (Black arrow) and in-between the muscle bundles (*). (Masson's trichrome ×40-Scale bar,50µm)

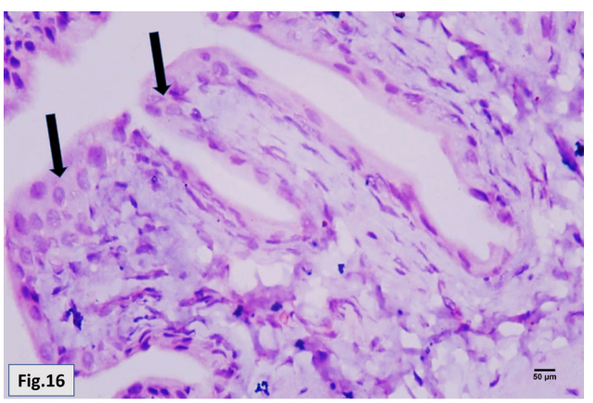


Fig. 16: A photomicrograph of group I rat's urinary bladder section showing negative cytoplasmic immune reaction for NF-kB of most epithelial cells (black arrows). (NF-kB x400-Scale bar,50µm)

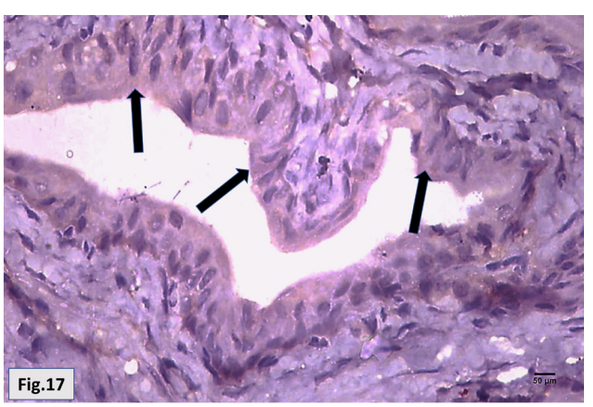


Fig. 17: A photomicrograph of group III rat's urinary bladder section showing strong cytoplasmic immune reaction for NF-kB of most epithelial cells (black arrows). (NF-kB x400-Scale bar,50µm)

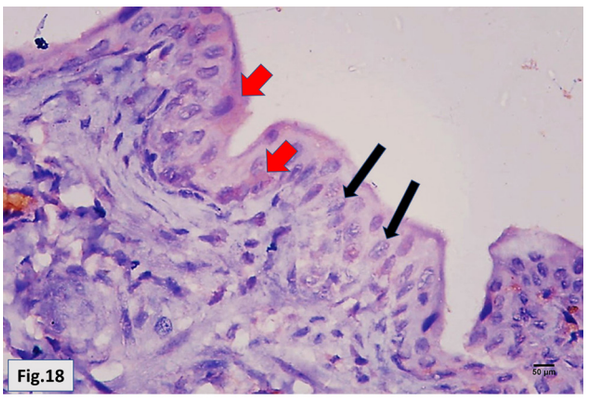


Fig. 18: A photomicrograph of group IV rat's urinary bladder section showing negative cytoplasmic immune reaction for NF-kB of most epithelial cells (black arrows). Notice, very few cells showing weak positive immune reaction (red arrows). (NF-kB x400-Scale bar,50µm)

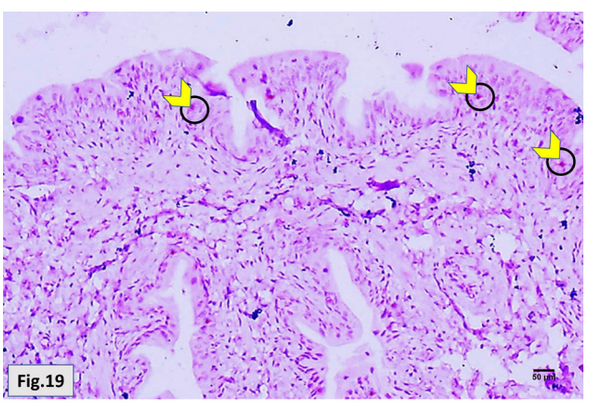


Fig. 19: A photomicrograph of group I rat's urinary bladder section showing positive immune reaction for PCNA of sparse basal nuclei of the epithelium (yellow arrowheads) (PCNA x100-Scale bar,50µm)

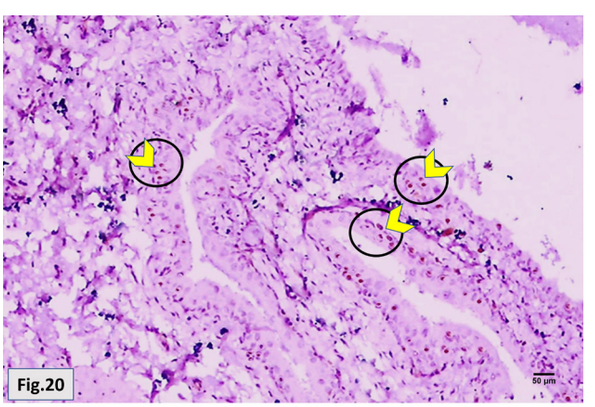


Fig. 20: A photomicrograph of group III rat's urinary bladder section showing positive immune reaction for PCNA of multiple nuclei among the epithelium (yellow arrowheads) (PCNA x100-Scale bar,50µm)

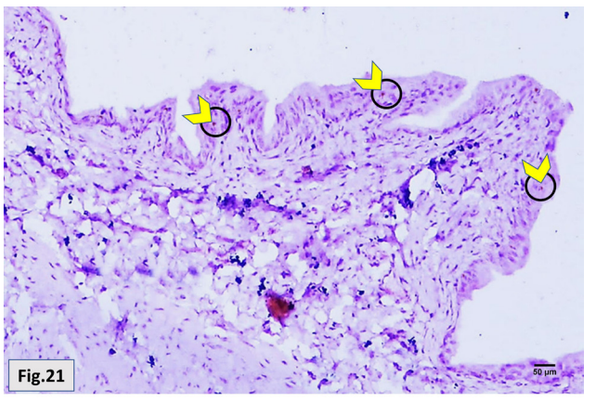
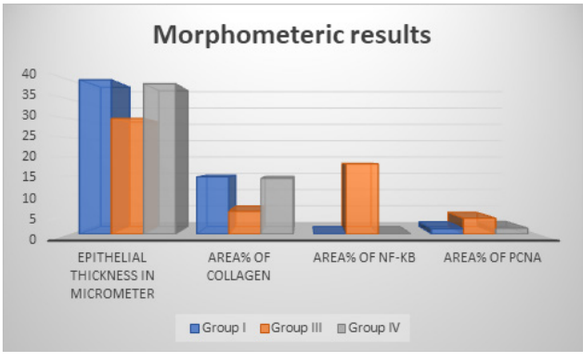


Fig. 21: A photomicrograph of group IV rat's urinary bladder section showing positive immune reaction for PCNA of sparse basal nuclei of the epithelium (yellow arrowheads) (PCNA x100-Scale bar,50µm)

Table 1: displaying the morphometric comparison between the groups I, III and IV. P-value highly significant (*) and nonsignificant (**).

	Epithelial thickness in µm	Area% of collagen	Area% of NF-κB	Area% of PCNA
	Area% (Mean ± Standard deviation)			
Group I	40.00 ± 2.0	14.8±0.1	0.02 ± 0.0	1.4 ± 0.1
Group III	30 ± 2.2	5.9 ± 1.2	18.2 ± 8.0	4.1 ± 0.8
Group IV	39.65 ± 0.7	14.4 ± 0.4	0.05 ± 0.0	1.5 ± 0.2
	(*P-value* between groups)			
Groups I& III	0.00001*	0.00001*	0.00001*	0.00001*
Groups I& IV	0.5**	0.1**	0.08**	0.2**
Groups III & IV	0.00001*	0.00001*	0.00001*	0.00001*



Histogram 1: displaying the morphometric comparison between the groups I, III and IV

DISCUSSION

Bladder toxicity incidence with cyclophosphamide treatment has been reported in up to 30% of patients[20] with the commonest form is acute cystitis that is characterized by hematuria in absence of infection and dysuria, and in severe cases it leads to obstructive uropathy[21,22]. Cystitis occurs within two days from the start of cyclophosphamide treatment, but late cystitis up to a month after stoppage of cyclophosphamide may occur[21,23].

Plenty of fluids, frequent voiding and bladder irrigation are recommended with cyclophosphamide treatment, to lessen the time of contact of its metabolites with the bladder mucosa[22]. In urinary bladder irrigation the bladder is flushed with sterile liquid then evacuated via a catheter, the process needs to be repeated over few days[24].

The role of intravesical injection of hyaluronic acid in ameliorating cyclophosphamide toxic effects on the urinary bladder of the adult male rats was histologically evaluated and confirmed morphometrically and statistically in the present study. Cyclophosphamide administration for 14 days showed marked changes of rats' urinary bladders in the form of lost mucosal folds, focal ulcerations, decreased epithelial thickness, degeneration of the epithelial cells with inflammatory reaction in the lamina propria, decreased collagen content. Widely separated smooth muscle bundles with degeneration of their cells and cellular remains in the lumen were also detected. These findings agreed with Cox[25] and Amanat *et al*[26] who reported that cyclophosphamide administration in rats induced bladder inflammation, hemorrhage and ulceration.

Cyclophosphamide cytotoxicity is attributed to its metabolites especially acrolein which is renally excreted active metabolite. Contact of cyclophosphamide metabolites with the bladder mucosa induce oxidative stress through production of reactive oxygen radicals, decreasing the activity of antioxidant enzymes and increasing lipid peroxidation that eventually lead to neutrophil infiltration, bladder inflammation and cell death, in addition to production of TNF-α that induce cell death via necrosis and apoptosis[27-31]. Its toxic effects on the cells are also achieved through alkylation of guanine groups of DNAs which prevents the double strands from uncoiling and separation with subsequent prevention of cellular multiplication followed by cellular apoptosis[4,5]. Wang *et al*.[32] attributed bladder dysfunction with cyclophosphamide administration in rats to the disturbance in smooth muscle / collagen ratio as cyclophosphamide led to decrease in the collagen fibers of the connective tissue.

Additionally, in the present study the urinary bladders of the rats that received cyclophosphamide showed marked increase in NF-κB and PCNA expression in the epithelial cells. Nuclear factor κB is an inactive cytoplasmic protein complex. Its activation induces several proinflammatory cascades through cytokines and chemokines release e.g., TNF-α, IL-1 and IL-6 thus performs a crucial part in the pathogenesis of the inflammation processes. Its significant increase in the urothelium was shown to be associated with cystitis in rats.

Proliferating cell nuclear antigen (PCNA) 'non-histone nuclear protein' is used as a marker for cellular proliferation[33-35]. The urothelium is considered very stable with sluggish proliferative activity. Though, when exposed to damage associated with epithelial loss, the urothelial cells proliferative activity is markedly increased with increased PCNA expression[36-38].

In contrary, administration of cyclophosphamide concomitant with intravesical injection of hyaluronic acid for 14 days in the present study showed regular structure of rats' urinary bladders, folded intact mucosa with tightly packed epithelial cells, slightly inflamed lamina propria, regularly separated smooth muscle bundles, proper amount of collagen fibers, mild expression of κB and PCNA in the epithelial cells.

Hyaluronic acid is a natural hydrophilic polymer, it was originally obtained from the animal tissues but due to the risk of immune response and diseases transmission, chemoenzymatic synthesis has been developed[39].

The superficial layer of epithelium of the urinary bladder contains dome shaped cells with their tight junctions contribute to the urothelial barrier along with the covering glycosaminoglycans layer that is secreted by the urothelium. The components of the glycosaminoglycans include hyaluronic acid. This layer protects the bladder wall from urinary solutes and microorganisms. Deficiency of hyaluronic acid in the glycosaminoglycans layer destroy the barrier. In bladder inflammation, damage of this barrier occurs increasing the cellular permeability and allows the hazardous substances to enter the bladder wall, inducing inflammatory reactions that lead to further damage of the urothelium and of the bladder wall[40,41].

Limited data have been reported regarding the effect of intravesical hyaluronic acid injection on the urinary bladder. However, Kallestrup *et al*.[42] reported an improvement of symptoms in 65% of female patients with interstitial cystitis after intravesical hyaluronic acid injection. Also, in another study using intravesical hyaluronic acid for refractory painful bladder syndrome, hyaluronic acid proved to be effective, well tolerated, and safe for all patients[43].

Moreover, in previous studies on rat models of induced cystitis by protamine and by E coli, intravesical injection of hyaluronic acid decreased bladder infiltration by inflammatory cells and preserved its antioxidant enzymes levels[44,45].

Hyaluronic acid protective mechanisms on the urinary bladder could be attributed to its ability to maintain the integrity of the bladder mucosal barrier and to coat the uroepithelium protecting it from cyclophosphamide metabolites, in addition to its antioxidant, anti-inflammatory and antiapoptotic effects[46-49].

Hyaluronic acid protects against cellular oxidative damage and modulates tissue hydration and osmotic balance which preserve the hydration and stability of the extracellular matrix where cells and collagen fibers are firmly maintained. Regarding its anti-inflammatory effect, hyaluronic acid acts as a signaling molecule interacting with cell surface receptors and with specific cytokines, Hence, it modulates the immune response, reducing the inflammation and cell death[50].

CONCLUSION

Intravesical injection of hyaluronic acid ameliorates cyclophosphamide toxic effects on the urinary bladder of adult male rats.

CONFLICT OF INTERESTS

There are no conflicts of interest.

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الملخص العربى

فاعلية حمض الهيالورونيك ضد التأثيرات السامة للسيكلوفوسفاميد على المثانة البولية لذكر الجرذ الابيض البالغ (دراسة نسيجية وكيميائية مناعية)

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المقدمة: سيكلوفوسفاميد هو دواء فعال مضاد للأورام يستخدم على نطاق واسع. سيكلوفوسفاميد له العديد من الآثار الجانبية للجهاز البولي والتناسلي بما في ذلك التهاب المثانة. يستخدم حمض الهيالورونيك في العديد من أنواع التهاب المثانة مثل التهاب المثانة الناجم عن الكيتامين والتهاب المثانة الخلالي لانه معروف بأنه جيد التحمل وآمن سريريًا.

الهدف: يهدف هذا العمل إلى تقييم دور الحقن داخل المثانة لحمض الهيالورونيك في تخفيف التأثيرات السامة للسيكلوفوسفاميد على المثانة البولية لذكر الجرذ الأبيض البالغ.

المواد والطرق: تم استخدام أربعين من ذكور الجرذان البيضاء تتراوح أعمارهم بين ٦-٨ أشهر واوزانهم تتراوح بين ٢٠٠-٢٥٠ جم. تم تقسيم الجرذان بشكل إلى أربع مجموعات (١٠ جرذان لكل مجموعة):

المجموعة الأولى (المجموعة الضابطة): تم تقسيمها إلى مجموعتين فرعيتين متساويتين:

- المجموعة الفرعية Ia لم تتلق الجرذان فى هذه المجموعة الفرعية سوى الطعام والماء لمدة اربعة عشر يوما.
- المجموعة الفرعية Ib تلقى كل جرذ حقنة واحدة داخل الصفاق من محلول ملحي ٠,٥ مل يوميا لمدة اربعة عشر يوما.

المجموعة الثانية (HA): تلقى كل جرذ حقن داخل المثانة بمقدار ٠,٥ مل من حمض الهيالورونيك يوميًا لمدة اربعة عشر يوما.

المجموعة الثالثة (CP): تلقى كل جرذ حقنة داخل الصفاق بمقدار ٣٠ مجم / كجم من سيكلوفوسفاميد يوميا لمدة اربعة عشر يوما.

المجموعة الرابعة (HA+CP): تلقى كل جرذ حقنة داخل الصفاق من سيكلوفوسفاميد كما في المجموعة الثالثة مع حقن حمض الهيالورونيك داخل المثانة كما في المجموعة الثانية.

النتائج: تسبب اعطاء سيكلوفوسفاميد حدوث تغيرات نسيجية ملحوظة في المثانة البولية للجرذان في شكل طيات مخاطية مفقودة ، وتقرحات بؤرية ، وتفاعل التهابي في الصفيحة المخصوصة ، وقلة الياف الكولاجين ، بينما اظهر اعطائه المصاحب بحقن حمض الهيالورونيك داخل المثانة بنية نسيجية منتظمة للمثانة؛ غشاء مخاطي سليم والتهاب طفيف في الصفيحة المخصوصة و كمية طبيعية من الياف الكولاجين.

الخاتمة: حقن حمض الهيالورونيك داخل المثانة يخفف من التأثيرات السامة للسيكلوفوسفاميد على المثانة البولية لذكر الجرذ الأبيض البالغ.